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Differential root morphology response to no versus high phosphorus, in three hydroponically grown forage chicory cultivars

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Abstract

Forage chicory is a productive forage resource for eastern North America; however, many soils in the region are acidic and deficient in P and might restrict the widespread use of forage chicory. There is no published information on response of forage chicory to P, or P acquisition strategies for morphologically different chicory cultivars. The literature suggests the following null hypothesis: "specific root length (SRL) will increase with P deficiency". We conducted controlled environment experiments using nutrient culture to determine plant mass, mineral composition, and root morphology of three forage chicory cultivars (Grasslands Puna (GP), LaCerta (LC) and Forage Feast (FF)) as a function of P supply, and test the null hypothesis with chicory. Phosphorus increased chicory growth irrespective of cultivar. Root morphology differed among cultivars independent of P supply with FF producing about twice the taproot mass of GP or LC. Root morphology was also impacted by P supply and the specific interactions between P and cultivar. Total root length and surface area of GP increased, and did not change in LC or FF under −P conditions. Thus, the null hypothesis must be rejected. Results suggest at least two different plant responses to −P conditions in chicory that seem to be attributes of specific cultivars: (a) increase in root length of the 0.28 mm root class (GP); (b) decrease in non-taproot mass density with −P and no change in root length or ratios between diameter classes (LC). The change in root length of small diameter class roots, as observed for GP, is typical of the responses to −P described in the literature. The decrease in root density seen with LC is probably an anatomical response that is not coupled with any observable morphological response. We conclude that use of the above null hypothesis as a paradigm for plant root response to P deficiency must be rejected. The routine use of specific root length as an indicator of environmentally induced changes in root system function is precluded by the presence of anatomical and physiological changes (adaptations) that have no concomitant gross morphological changes.

Keywords: *Cichorium intybus* L.; Cultivars; Root length; Root diameter; Specific root length; Root density

1. Introduction

Forage chicory is a very productive plant for pasture in eastern North America (Belesky et al., 1999, 2000; Collins and McCoy, 1997; Kunelius and MacRae, 1999). However, many soils in the region are acidic and deficient in phosphorus (P) and calcium (Ca) and might restrict the optimal growth of forages that respond to nutrient inputs and have high nutrient content, such as chicory (Belesky et al., 2001). Lime and fertilizer P help meet crop nutrient requirements; however, some plants grow well in marginally fertile soils because of mechanisms facilitating efficient P uptake even when the apparent P supply in the soil is modest (Marschner, 1991).

Changes in root morphology in response to changes in P availability have been shown to be an important factor in P efficiency for a number of different species from different functional groups (e.g., rape, *Brassica* sp., bean, *Phaseolus* sp., maize, *Zea mays* L., buckwheat, *Fagopyrum esculentum* L.). The most notable response is a more rapid root development, higher root shoot ratio, and finer and longer roots and root hairs (Amann and Amberger, 1989; Elliott and Lauchli, 1985). These changes can be expected to improve P acquisition and facilitate an exploration of a greater soil volume (Marschner, 1986; Fusseder, 1987). Other species, however, have not shown these responses

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when treated to differential P levels (e.g., *Brachypodium pinnatum* (L.) Beauv. and *Dactylis glomerata* L.; Ryser and Lambers, 1995; Ryser, 1998). This lack of response may have been due to experimental conditions, or, alternatively, to specific species/cultivar response patterns.

Root morphological studies on soil grown plants are notoriously imprecise, especially with the finest roots (Pallant et al., 1993). In addition, P studies with unsterilized soil are subject to confounding by endemic mycorrhizal infections. An alternative to soil for studies of root responses is the use of hydroponics or aeroponics to grow plants with easy access to all of the roots (Waisel, 2002). In classical hydroponics, a boundary layer develops around the active absorbing regions of the root, modifying apparent uptake kinetics of even the most mobile ions (Weathers and Zobel, 1992). Hydroponics with vigorous aeration, thin film (nutrient film technique, NFT) hydroponics and aeroponics, obviate this problem, giving the root immediate access to even the least mobile ions. We know of no studies that have quantified P availability vis a vis aeration levels (boundary layer thickness). We would not expect P mobility through a boundary layer in hydroponics to be any less rapid than other ions of similar size and charge; as contrasted to P mobility in soil. Uptake of P, under aerated hydroponics conditions, would be so rapid, even at concentrations known to be deficient in soil, that only a P absent treatment would provide an adequate contrast to a P present treatment.

Based on published data with soil grown plants, an appropriate null hypothesis for studies of root system response to P deficiency in a hitherto un-investigated species would be: specific root length (SRL) will increase with P deficiency. We believe that a sensitive test of this hypothesis with a previously un-studied species would be to pre-culture seedlings in hydroponic solutions with normal hydroponic levels of P and then to transfer them to hydroponics with or without P. The −P seedlings will have adequate reserve P levels for several days, allowing them to respond to the absence of P for several additional days before internal concentrations begin to inhibit growth (Aono et al., 2001). To complete the test, at the end of two weeks of culture, measure and compare SRL and root and shoot biomass between the two treatments.

There is no published information on the response of forage chicory roots to P application. To test the above null hypothesis with this species, we conducted controlled environment experiments using aerated hydroponic culture to determine dry matter accumulation and partitioning, and root morphology of three forage chicory cultivars known to differ in root characteristics (Alloush et al., 2003; Sanderson and Elwinger, 2000).

2. Materials and methods

2.1. Plant cultivation

Commercially available chicory seeds (Cichorium intybus L., cv. Grasslands Puna (GP), LaCerta (LC) and Forage Feast (FF)) were germinated in cones containing potting mix (Professional PRO-MIX[1] growth medium, Premier Horticulture Inc., Red Hill, Pennsylvania). Twenty-four seedlings (8-day-old) from each cultivar were transferred into 1-L pots (one seedling per pot) containing complete half-strength nutrient solutions (pH 6.0) for a 3 day pre-culture interval. The hydroponic culture media was composed of a continuously aerated solution of: 1.5 mM $Ca(NO_3)_2$; 0.5 mM K_2HPO_4; 0.5 mM $MgSO_4$ (all at, roughly, 40% of Long Ashton Nutrient Solution recommendations; Hewitt, 1966—these levels and conditions were chosen to be comparable with the previous research by Neel et al., 2002) and micronutrients supplied according to Long Ashton formula (Hewitt, 1966). After pre-culturing, seedlings of similar mass were selected (1.708 ± 0.2 g) and treatments (+ or −P) were imposed in full-strength nutrient solution with continuous aeration. The solution for P-stressed plants (−P treatment, i.e., no P) had K_2HPO_4 replaced with 0.5 mM K_2SO_4 to achieve maximum differential response, assuming P carry-over from initial and pre-cultures (Aono et al., 2001). The pH of all solutions was adjusted to 6.0 using 1 M H_2SO_4 or NaOH. Nutrient solutions were renewed every other day and adjusted to 1 L with deionized water. Total number of pots (plants) was 24, three cultivars, two treatments, and four replicates per cultivar-treatment combination.

2.2. Harvest procedure

Plants were harvested 14 days after placement in treatments, shoots separated from roots and fresh weights recorded. Image analysis (Decagon Devices Inc., Agimage, Pullman, WA) was used to determine leaf number and area. Taproot volume was calculated from digital photographic images, assuming the taproot was a perfect cone ($v = 1/3\pi r^2 l$). Root diameter, length, and branching were measured using digital photographs and Rhizo™ software (Regent Instruments Inc., Canada) (see Zobel, 2003a, for root imaging and analysis methodology). Non-taproot volume was calculated assuming cylindrical root segments ($v = \pi r^2 l$).

After photographing, dried shoots and roots were ground to pass a 0.5 mm screen and kept in sealed plastic bags prior to mineral analysis. Plant tissues (50–100 mg) were weighed into Teflon containers, 1.0 mL of 15.8 M HNO_3 added and heated in a microwave (Kingston and Jassie, 1988). Digested solutions were brought to a final volume of 10 mL with distilled, deionized water. Solutions were filtered and stored in plastic tubes at 5 °C until analysis for P, S, K, Ca, Mg, and Na using inductively-coupled plasma spectroscopy (Jobin Yvon Model JY 46P ICP, Longjumeau, France). Total nitrogen in shoot and root tissues was determined by Carlo Erba analyzer (EA 1108 CHNSO, Milan, Italy).

[1] Trade names are for the convenience of the reader and do not imply endorsement by USDA.

Table 1
Mean values and their *F*-test significance levels for shoot characteristics, root mass and shoot and root phosphorus concentration

Cultivar	P treatment	Shoot			Total root				Taproot		
		Dry-mass (gm)	Specific leaf area ($m^2\ gm^{-1}$)	Phosphorus ($\mu g\ gm^{-1}$)	Phosphorus ($\mu g\ gm^{-1}$)	Fresh-mass (gm)	Dry-mass (gm)	Dry-mass fresh-mass^{-1}	Fresh-mass (gm)	Dry-mass (gm)	Dry-mass volume^{-1} ($gm\ cm^{-3}$)
GP	−P	1.23	98.3	710	713	8.38	1.65	0.204	1.15	0.222	0.315
GP	+P	3.14	164.3	6802	9111	18.84	2.24	0.120	3.52	0.421	0.354
LC	−P	1.14	85.0	677	842	6.65	1.49	0.227	0.94	0.219	0.509
LC	+P	2.78	148.8	10055	12505	18.43	2.06	0.112	2.68	0.298	0.237
FF	−P	1.33	93.5	744	733	9.19	1.93	0.210	2.34	0.492	0.313
FF	+P	2.97	135.6	6041	6360	16.34	2.44	0.150	6.41	0.970	0.201
	LSD05	0.47	45.7	1488	1336	3.22	0.35	0.032	1.04	0.200	0.199
Cultivar		–	***	**	***	–	**	–	***	***	–
P treatment		***	***	***	***	***	***	***	***	***	*
Interaction		–	**	***	***	*	–	*	**	**	*

(–) Not significant.
* Significant at $P<0.05$.
** Significant at $P<0.01$.
*** Significant at $P<0.001$.

2.3. Data analysis

Data were analyzed using the additive main effects and multiplicative interaction analysis (AMMI) procedure (Zobel et al., 1988; Zobel and Wallace, 1995). With this procedure, means are assessed pairwise against an LSD_{05}.

3. Results

3.1. Shoot and root growth

Phosphorus deficiency decreased chicory shoot mass and leaf area irrespective of cultivar (Table 1; Fig. 1). Phosphorus deficiency symptoms appeared on leaves of all cultivars after 6 days of growth, coinciding with the initiation of rapid increase in whole plant growth of the high P treated plants. The observed delay of 6 days in the separation of growth rates of −P versus +P plants confirmed the assumption of carryover from pretreatment conditions. Insufficient P reduced shoot more than root mass (Table 1), consequently shoot to root ratios were less in −P compared to + P plants. Grasslands Puna specific leaf area (SLA) was greater than that of LC and FF with + P, but −P reduced SLA of all cultivars and did so the most for LC (Table 1). It was not determined if this was due to a change in leaf density and/or thickness. Phosphorus concentrations in the roots and shoots varied with cultivar, treatment, and interaction between the two (Table 1). Grasslands Puna and FF had nearly 10-fold decreases in root and shoot P concentration with −P, while LC had nearly a 15-fold decrease. Contrary to expectations, the patterns of observed variance and interactions with P concentration do not correlate with any of the variance patterns of the measured morphological characteristics, including dry matter accumulation (multivariate analysis not presented).

Total root fresh-mass was not different between cultivars, but increased with the +P treatment and the magnitude of the +P effect differed among cultivars (Table 1). Total root dry-mass differed with cultivar and P treatment, but these effects were independent (Table 1). On the other-hand, the ratio of total root dry-mass to fresh-mass decreased with +P treatment, the decrease in this estimate of tissue density was much

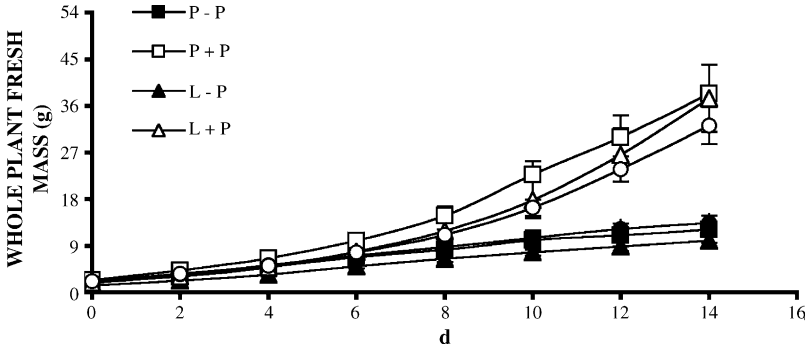

Fig. 1. Effect of P supply on fresh-mass of chicory Puna (P), LaCerta (L), and Forage Fest (FF) cultivars grown in nutrient solution. Bars are standard errors of means ($n=4$).

Table 2
Non-taproot means and significance levels, and selected diameter class lengths

Cultivar	P treatment	Non-taproot				Diameter class (cm)				
		Total length (cm)	SRL (cm gm^{-1})	Average diameter (mm)	Dry-mass volume^{-1} (gm cm^{-3})	0.10	0.28	0.57	0.86	1.10
GP	−P	1913	13.57	0.316	0.955	164	1473	243	25.3	6.76
GP	+P	1345	7.36	0.372	1.288	100	914	263	57.9	7.90
LC	−P	1690	13.35	0.311	1.007	197	1244	203	34.3	8.28
LC	+P	1810	10.61	0.313	1.276	214	1292	258	33.4	7.88
FF	−P	1142	8.10	0.379	1.124	90	731	255	44.0	13.61
FF	+P	1567	10.85	0.337	1.118	195	1070	248	43.3	7.22
	LSD05	433	3.74	0.053	0.215	145	372	87	18.5	8.37
Cultivar		*	–	*	–	–	*	–	–	–
P treatment		–	–	–	**	–	–	–	*	–
Interaction		**	**	*	*	–	**	–	*	–

(–) Not significant.
* Significant at $P<0.05$.
** Significant at $P<0.01$.

less for FF than the other two. Taproot volume and fresh-mass differed between cultivars and increased with +P treatment. Forage Feast had more taproot fresh-mass under −P and increased two to three times as much with +P (Table 1). Taproot dry-mass of GP and FF increased with +P, similar to the fresh-mass, while LC did not change taproot dry-mass as a result of P treatment. Taproot dry-mass of GP and LC averaged 7% of total plant dry-mass, while FF taproot dry-mass was 15% of total under −P and 18% of total under +P treatments. The taproot dry-mass to volume ratio (mass density, MD) did not change with GP or FF, but that of LC increased significantly with −P.

3.2. Root morphological changes

Total root length was different between cultivars. Cultivar by treatment interaction effects are also present since P treatment effects increased GP, decreased FF and did not significantly impact LC total root lengths (Table 2). Specific root length was influenced by P level through differential effects on the cultivars. The SRL pattern of variation mimicked the total root length pattern for GP and FF, but tended to reverse with LC (Table 2). Branch number differed among cultivars in a pattern similar to total length (data not shown). Grasslands Puna at −P had smaller diameter roots with greater total length and greater branching than +P, resulting in greater SRL in −P GP. On the other hand, FF had larger diameter roots with less total root length and fewer branches and decreased SRL for −P compared to +P. Phosphorus treatment had minimal influence on these root characteristics for LC. The ratio of non-taproot dry matter to non-taproot volume (non-taproot MD) indicates that the density of GP and LC roots decreased with −P compared to +P, but did not differ in FF.

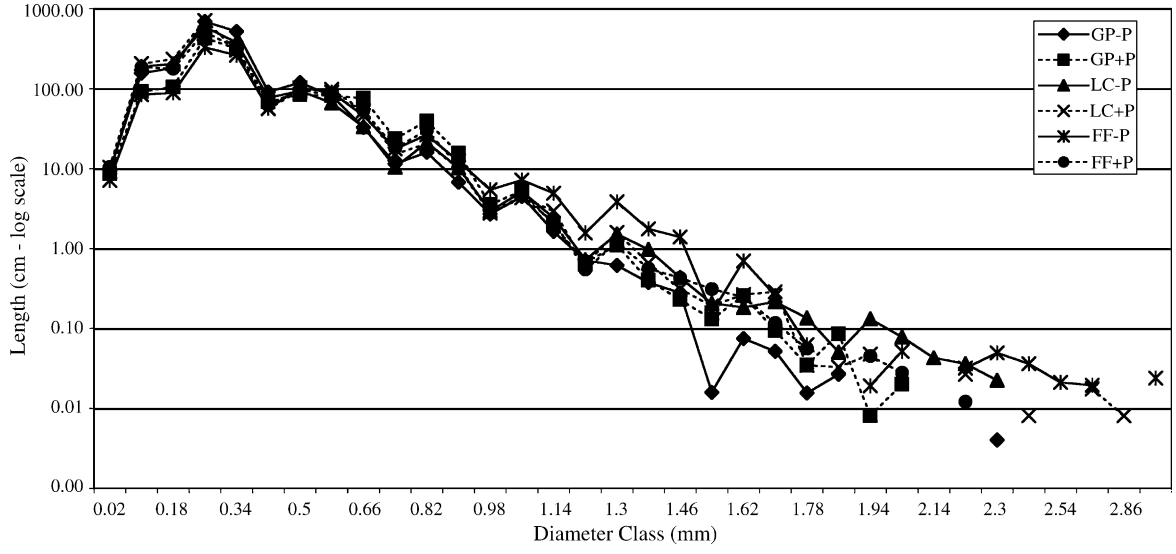

Fig. 2. Log_{10} of the measured diameter class lengths of the different treatment/cultivar combinations plotted against the diameter classes.

Table 3
F-test significance tables of diameter class length and nutrient contents

	Diameter class																	
	0.02	0.10	0.18	0.26	0.34	0.42	0.50	0.58	0.66	0.74	0.82	0.90	0.98	1.06	1.14	1.22	1.30	1.38
Cultivar	–	–	–	*	*	*	–	–	–	–	–	–	–	–	–	–	–	–
P level	–	–	–	–	–	–	–	–	*	–	*	–	–	–	–	–	–	–
Interaction	–	–	*	**	*	–	–	–	–	–	*	*	–	–	–	–	–	–

	Shoot nutrients											
	N	S	Mg	Na	K	Ca	Mo	Mn	Fe	Zn	Cu	B
Cultivar	***	**	*	**	–	–	–		–	–	–	*
P level	***	–	**	–	***	–	–	*	*	***	*	–
Interaction	*	–	–	–	***	–	–	***	–	–	–	–

	Root nutrients											
	N	S	Mg	Na	K	Ca	Mo	Mn	Fe	Zn	Cu	B
Cultivar	***	***	***	**	–	***	–	***	*	–	–	***
P level	***	***	***	***	–	***	***	***	–	–	–	***
Interaction	*	**	***	***	*	–	*	***	–	–	–	–

(–) Not significant.
* Significant at $P < 0.05$.
** Significant at $P < 0.01$.
*** Significant at $P < 0.001$.

Root length and diameter was measured from digital photographs with a resolution of 12.12 pixels per mm (p mm^{-1}). The resulting diameter class length plot (DCL) shows distinct peaks when length is plotted on a log scale (Fig. 2). Assuming these peaks are the result of discrete root diameter classes and not artifacts (Zobel, 2003a), the DCL data can be reduced to nine diameter classes (DC) of 0.1, 0.28, 0.57, 0.86, 1.1, 1.36, 1.68, 1.99, and 2.31 corresponding to the midpoints of the peaks. Analysis of variance of the original data (Table 3) supports this grouping, since classes combined into the 0.28 DC had significant cultivar and interaction variance but no significant P treatment variance, while those combined into the 0.86 DC have significant P treatment variance but no significant cultivar variance (Table 3). The 0.28 DC constitutes 50–70% of the total root surface area per plant (Fig. 3) and the 0.57 DC an additional 20–35% so that the two combined with the 0.86 DC make up 99% of the total root length (Fig. 3).

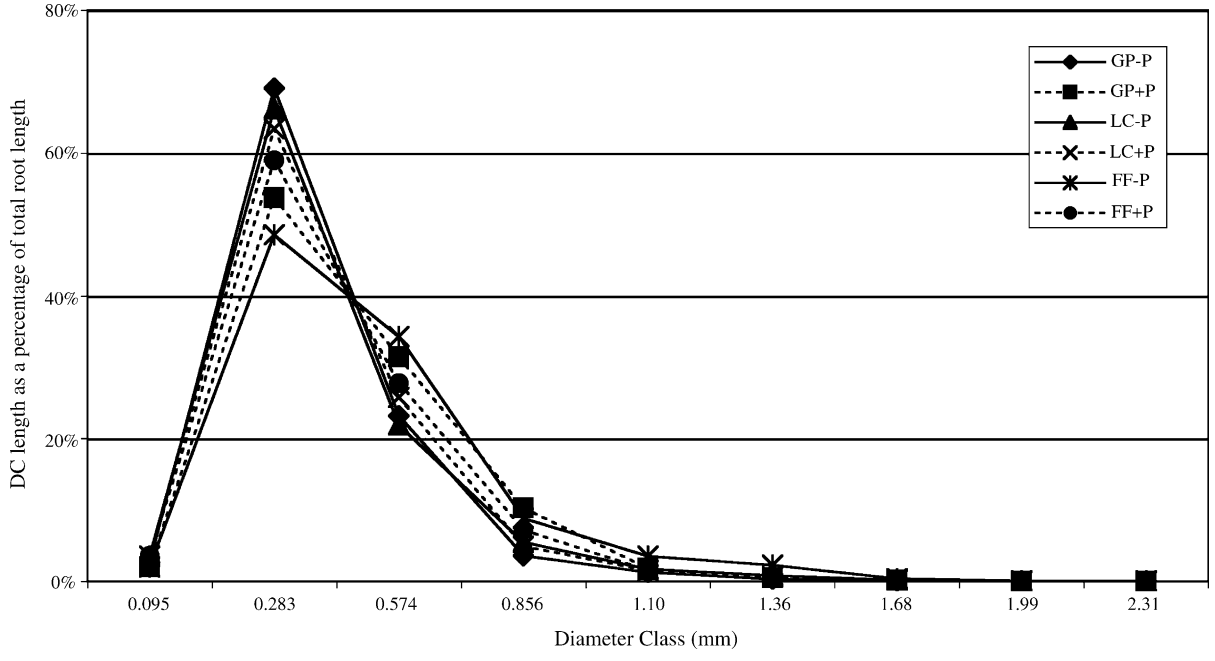

Fig. 3. Relative proportion (%) of total root surface area represented by the different diameter classes of the chicory roots under + and −P.

The 0.28 DC lengths not only differed, significantly, between cultivars irrespective of P, they also differed between cultivars in response to P treatment (i.e., show interaction, Table 2, and the strongly opposite "cross-over" patterns between 0.28 and 0.57 DC roots of GP and FF, Fig. 3). The 0.28 DC showed a response to treatment pattern identical to total root length. In the 0.86 DC, only GP responded to P treatment, and this was to increase with +P. None of the other DC had significant responses to P treatment, nor did they differ significantly between cultivars.

3.3. Mineral nutrient concentrations

Shoot concentrations of K, Mg, and N decreased, Na, S, and Ca did not change and Cl increased under −P conditions (data not shown). Nitrogen, S, Mg, Na, and B varied with cultivar and N, Mg, K, Mn, Fe, Zn, and Cu varied with P treatment (Table 3). Concentrations of all nutrients, except Na, were less in roots than in shoots (comparatively little Na was translocated to the shoot). Zinc, Mo, and Cu concentrations in roots were similar among cultivars, whereas P treatment did not influence Fe, Zn, or Cu concentrations. Similar to the shoot, roots under −P treatment had increased levels of Cl. Potassium increased with +P in GP and LC, but decreased in FF ($GP-P=52\,mg\,kg^{-1}$; $GP+P=70\,mg\,kg^{-1}$; $LC-P=60\,mg\,kg^{-1}$; $LC+P=68\,mg\,kg^{-1}$; $FF-P=62\,mg\,kg^{-1}$; $FF+P=49\,mg\,kg^{-1}$) giving rise to significant interaction without significant additive variance. None of these nutrient variance patterns correlated to any of the morphological variance patterns.

4. Discussion

Overall growth patterns of chicory, in response to + or −P, were similar to those reported for other plant species subjected to −P conditions (Hoffland et al., 1990; Le Bot et al., 1990; Amann and Amberger, 1989), although specific cultivar responses varied. Dry weight P concentrations are much higher in the +P treatments (e.g., $12.5\,mg\,gm^{-1}$, Table 1) than reported for some field grown chicory plants (e.g., $2\,mg\,gm^{-1}$; Améziane et al., 1997; Schittenhelm, 1999). Since chicory is a mineral accumulator (Belesky et al., 2001) the relatively high P concentration in Long Ashton Solution and the use of substrates without P binding capacity may have led to luxury P uptake here and in a previous experiment (Neel et al., 2002). Leaf area was influenced by −P conditions, as were leaf number, and specific leaf area. It is not possible to conclude whether differences in SLA were due to changes in leaf density or leaf thickness, or both. Both occur in other species relative to cultivar differences in SLA (Lugg and Sinclair, 1979). Total root fresh-mass and dry-mass was greater under +P compared to −P, as expected. The dry-mass to fresh-mass ratio was less under +P, suggesting a decrease in total root density under that more optimal growing environment. Ryser (1996) and Wahl and Ryser (2000) associate lower tissue densities with faster growth rates and shorter root life spans. They also suggest that plants normally adapted to a more nutrient rich environment have lower tissue densities.

Forage Feast is believed to have been selected for its taproot size. It has twice the taproot fresh-mass of the other two cultivars under +P and nearly three times the dry-mass. The change from 15% to 18% of total dry-mass for FF with +P suggests that taproot dry-mass increases at the expense of other root tissues. The dry-mass to volume ratio of FF and GP remains constant, but for LC the fresh-mass of the taproot increases with +P compared to −P, while the dry-mass is constant and the MD of the taproot doubles under −P. Apparently, LC adapts to the different P treatments by changing tissue density (decreasing volume) of the taproot without changing total taproot dry-mass. The greater density of the LC taproot under −P suggest that this cultivar may normally encounter low P conditions in the region from which it was originally selected (for related comments, see Ryser, 1996, 1998).

Current technology (Zobel, 2003a,b), which allows diameter measurement of roots less than 0.01 mm in diameter, allowed us to collect data that demonstrate the existence of nine diameter classes of root in 22-day old chicory plants. The generalization, from the literature, that less P results in thinner, longer roots (increased SRL) is not supported by these data. Total root length of the 0.28 DC of GP increased under −P as expected from the literature, but that of LC was unchanged and FF tended to decrease (Table 2).

Root length and branching is correlated with the combined changes in the 0.28 and 0.86 DC, which usually are short (Cahn et al., 1989; Paollilo and Zobel, 2002), so changes in total class length are linked to changes in branching. Specific root length may indicate root function (Fitter, 1985; Eissenstat et al., 2000) based on its tracking of decreases in average root diameter which are assumed to relate to increases in nutrient and water uptake. Specific root length is the ratio between non-taproot total root length and non-taproot total root dry-mass. Since volume changes as root length and diameter change, SRL can be expected to change as average diameter changes (assuming constant density). The constant density assumption does not hold with this chicory data. The apparent density of the non-taproot portion of the LC and GP root systems decreased with −P treatment while that of FF did not change. Ryser and Lambers (1995) suggest that lower root density translates to higher metabolic activity. Wahl and Ryser (2000) suggest that a decrease in root density can occur as a result of thinner cell walls in the stele or a decrease in the stele to cortex diameter ratio, either of which could translate to more rapid mineral uptake. LaCerta did not change in root morphological characteristics under −P, but did change to lower density non-taproots, thus suggesting increased P uptake activity. Forage Feast, on the other hand had no responses that clearly suggest any non-taproot adaptation to the −P treatment.

The lack of a consistent response of the 0.28 DC roots to −P, coupled with the apparent changes in density of LC

non-taproots, suggests two differing response mechanisms for chicory in low P situations: (1) increases in length of small diameter roots (increased surface area for absorption) and (2) decreased root density (increased metabolic activity). The apparent lack of a typical response to −P by FF is probably due to the high sink strength of the taproot, although we cannot rule out a purely physiological change in P uptake efficiency.

Actual P uptake did not correlate to any of the measured variables, including SRL. We suggest that this lack of correlation is an artifact of the experiment conditions. Hydroponics, with aeration, provides the plant with an effectively mobile form of P that is acquired easily through mass flow, rather than the rather steep diffusion and concentration gradients that occur in soil. Therefore, root morphology and growth rate are not as important to control of P uptake under these conditions as internal physiological regulatory factors.

5. Conclusions

Our results suggest that chicory, as a species, has at least two inherent patterns of response to low or zero phosphorus conditions. One pattern is the classical increase in the length of the smallest diameter roots in response to P deficient conditions. The second pattern is a significant decrease in root tissue density, and therefore increased activity, under −P conditions. Since only one of the tested cultivars followed the pattern suggested by the null hypothesis, we must reject that hypothesis as a paradigm for plant response to P deficiency. The data presented demonstrate that there are three groups of roots in chicory each with independent response patterns: the 0.28 mm diameter class, which comprises nearly 90% of total root length; the 0.86 mm diameter class which increases in length in GP with the addition of P; and all the rest. The differential response patterns of the three groups of roots and the observed change in density of the roots, suggests that the routine use of SRL for monitoring differences in root system function should be reconsidered. This data provides a theoretical basis for developing hypotheses about Chicory root system responses to P treatments that can be tested under soil conditions.

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